

ART. XIX.—*On Chlorophyll, Carotin and Xanthophyll, and on the Production of Sugar from Formaldehyde.*

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In a previous paper the theory was put forward that the production of carbohydrates in plants did not take place by a direct synthesis of carbon dioxide and water to form formaldehyde, and then sugars by polymerization, but that the carbon dioxide and water combined with the phytol base of chlorophyll to form xanthophyll or carotin, and that this by photo-oxidation produced formaldehyde, reducing sugar and phytol, the latter recombining with the chlorophyll molecule. This would represent a change in which chlorophyll played the part of an enzyme, requiring a supply of energy in the form of light for its activity. This conclusion was mainly based upon the facts that formaldehyde is produced by the photo-oxidation of chlorophyll films in the presence or absence of carbon dioxide, and that chlorophyll films in contact with water saturated with carbon dioxide turn first yellow and then brownish white, slowly in darkness and rapidly in light, while at the same time they appeared to gain in weight and did not set free any oxygen.

In the earlier work I was able to extract small amounts of xanthophyll from chlorophyll films decomposed by carbon dioxide by treatment with potash and extraction with alcohol and separation with ether. Hence the conclusion was made that an actual production of xanthophyll had taken place. Jürgensen and Kidd¹ suggest, however, that the chlorophyll films used might have contained xanthophyll as an impurity, and they confirm Willstätter's² statement that the action of carbon dioxide on chlorophyll is due to its removing magnesium, and converting the chlorophyll into phaeophytin, which in the form of films is yellow in colour.

On using chlorophyll separated from 80 per cent. acetone by petrol ether, and removed from the latter, after washing, by the

1 Proc. Roy. Soc., January, 1917, p. 342.

2 Sitzungsber. d. Kgl. Preuss. Akad. der Wiss., 1915, pp. 322, 544.

addition of tale and filtering, no xanthophyll could be extracted from the films decomposed by carbon dioxide.

Nevertheless, that the first stage in photo-synthesis might be a direct addition synthesis with carbon dioxide is by no means impossible. It has long been known that direct addition syntheses of carbon dioxide can be produced with the aid of strong reducing agents such as sodium or potassium (Wanklyn, Kolbe), Bremmer (Annalen, 350, 1906, p. 313) has shown that hydroquinone heated with potassium carbonate, bicarbonate, glycerine and sodium sulphate in a stream of carbon dioxide yields oxysalicylic acid and dicarboxylic acid ($C_8H_6O_6$). Similar results were obtained with phenol and resorcin, while gallic acid yielded a gallo-carboxylic acid. Presumably phenols may be transformed into Ketone combinations with a free affinity to which carbon dioxide can attach itself, that is a direct addition synthesis with carbon dioxide takes place.

The action of watery solutions of carbon dioxide on chlorophyll in light and in darkness.

Pure dry carbon dioxide does not appear to exercise any action upon chlorophyll films in darkness, or even in light, after several weeks' exposure the films being still green and not appreciably altered. As the presence of water is necessary, a large bulb tube was lined with chlorophyll half way from the bulb end. The tube was filled with pure carbon dioxide over mercury, and after introducing 0.5 c.c. of water saturated with carbon dioxide, was exposed to light for three weeks. The films were still green, but with a yellow tinge. The weight of chlorophyll used was 0.216 gram. Out of 2440 c.c. of carbon dioxide 15.6 c.c. disappeared, and the residual gas contained 0.4% of nitrogen, but no oxygen. Apparently a rapid reaction between chlorophyll and carbon dioxide only takes place when the former is in direct contact with a watery solution of the latter.

Repeating the experiment with a tube containing sufficient water to cover the film, the upper end being drawn out, sealed, and the tube exposed for one week, the water became pale yellow, and the chlorophyll yellowish green. Eight c.c. of saturated water were used, 40 c.c. of carbon dioxide and 0.22 gram of chlorophyll. After bringing the whole tube to the original temperature, and allowing for the pressure, the gas had decreased in volume only 3.8 c.c. (i.e., 0.007 gram CO_2 per 0.006 gram of Magnesium). On evaporating the water to dryness, and adding the chlorophyll film

after dissolving it in hot alcohol, the total weight of residue was 0.21 gram, i.e., a slight loss instead of a gain.

In the experiments mentioned in the previous paper in which chlorophyll films appeared to gain in weight after exposure to light in water saturated with carbon dioxide, the films were weighed in situ in the tube after pouring off the water and drying in a current of rarified dry air at room temperature. Repeating a similar experiment, a film weighing 0.105 gram appeared to increase in weight to 0.228 gram. Close observation showed, however, that dry chlorophyll films in water swell and imbibe water, and that this water is not removed wholly by drying at room temperature, the films assuming an approximately constant weight while still containing a large percentage of water. The same film on dissolving in alcohol and petrol ether weighed on drying 0.102 gram.

On the other hand, if dried in air at 50°C. or 60°C., the films lose weight by oxidation. In one test experiment this loss amounted to 3% in ten hours. In a second experiment a dry film weighing 0.264 gram, weighed 0.251 gram after sixteen hours at 60° in air and darkness, and 0.234 gram after forty hours, being then quite yellow with nearly all the green colour lost. This error can be avoided by drying in *vacuo*, or in hydrogen or nitrogen. In a further experiment conducted with every possible precaution, a chlorophyll film weighing 0.228 gram after one week's exposure to light in water saturated with carbon dioxide weighed 0.223 gram, i.e., it lost slightly instead of gaining in weight.

The film is, however, not wholly insoluble, but a little matter passes into solution in the water, and this may include one product, formaldehyde, which is mostly lost on evaporation.

Experiments on a large scale were then carried out by using ordinary sparklet syphons. Dry chlorophyll, obtained by petrol ether separation from an acetone extract and subsequent purification, was weighed in a small beaker, dissolved in a little ether, poured into the syphon, and the beaker then dried and reweighed. A current of warm air was passed through the syphon, which was inclined and revolved until an even chlorophyll film formed up to the danger mark. The syphon was then filled to this level with cold boiled water, the head screwed on a sparklet bulb attached, and after previous exhaustion by a Geryck pump while in the inverted position, the bulb was pierced, and the water charged highly with carbon dioxide. One syphon was exposed to sunlight for a week, the other left in darkness. Owing to the sunlight warming

and partially melting the chlorophyll, the film sometimes becomes more or less distorted. This can largely be avoided by keeping the syphon under cold water flowing from a tap. The syphons remained highly charged with carbon dioxide, but contained no oxygen.

The films on washing with absolute alcohol gave a yellow solution containing phaeophytin and turning green with zinc acetate. The water was also evaporated to dryness. It was at first clear and colourless, but became faintly brown on evaporating. A trace of solid residue was left by the water from both syphons, which was partly soluble and partly insoluble in water. It averaged under 2% of the weight of chlorophyll used.

If the films are prepared from the first petrol ether separation of chlorophyll without further purification, the water always leaves a larger residue, and that from the syphon exposed to sunlight always contains some reducing sugar. Thus, in one case, the water from the syphon in darkness left a residue containing a little matter soluble in water, and giving a trace of reduction by Fehling's test, and a brownish white waxy solid soluble in petrol ether. The residue from the syphon in sunlight contained more matter soluble in water, and gave a strong reduction with Fehling's test. Further tests showed a hexose sugar to be present. The weights were in grams.

	Sunlight.	Darkness.
Chlorophyll film - - - - -	0.359	0.37
After 1 week in water + CO ₂ under pressure -	0.265	0.306
Residue from evaporation of water - - -	0.056	0.039
Total Residue -	0.321	0.345

The same experiment was repeated, using thicker films of chlorophyll, exposures of four weeks' duration, and evaporating the large bulk of water under reduced pressure and temperature. The water from the sunlight syphon left a brown residue on the sides of the vessel on evaporating, and a gummy residue on the bottom. More than half of the residue was soluble, and it gave the tests for a reducing hexose sugar. The water from the syphon in darkness left a brown non-gummy residue of which less than 1/5 was soluble in water, and gave a white flocculent precipitate with Fehling's test, but no reduction. No trace of free oxygen was found.

For demonstration purposes the syphon can be filled half way above the danger mark and then charged with a sparklet, using a control without carbon dioxide. After a week in darkness a comparison of the two syphons forms an admirable lecture demonstration of the action of a solution of carbon dioxide on chlorophyll, the film in the first case being yellow, in the latter, green.

The film from sunlight was in the thinner parts nearly colourless, and in the thicker parts more brown than yellow. It contained less phaeophytin than the films kept in darkness. The weights were—

	Sunlight, grammes.	Darkness, grammes.
Chlorophyll film - - - - -	0.555	0.527
Total film residue after 4 weeks - - -	0.301	0.458
Total water residue - - - - -	0.205	0.058
Total - - - - -	0.506	0.516

From the above it would appear that in sunlight and CO_2 , chlorophyll decomposes slightly more than in darkness, that a little formaldehyde or other volatile product is formed, and that if any oxygen is formed, it is not set free, but oxidises the phaeophytin. The action is not continuous as in the plant, since there is no reconstruction of the chlorophyll.

It appears, further, that the sugar formed in the plant remains at first in loose combination with the chlorophyll, and follows it into the petrol ether and other solvents, and that it is only separated from the chlorophyll when this is purified by precipitation.

If pure chlorophyll films are dusted over with zinc dust, they remain green in contact with a solution of carbon dioxide in water indefinitely, and even after a week's exposure to sunlight only show a slight fading on the more exposed side. On dissolving the films the solution shows the same absorption bands as chlorophyll, but the solubility is altered.

On incinerating, the ash of the green pigment was found to consist of zinc oxide, and not of magnesium oxide. It evidently consisted of the salt of zinc with phaeophytin, described by Willstätter and Hocheder (*Annalen der Chemie*, 1907, 353, p. 205), which closely resembles chlorophyll. Evidently in the presence of a saturated solution of carbon dioxide in water, zinc will displace the magnesium of chlorophyll. In the previous paper I had described some results which seemed to show that with the aid of zinc dust and in the presence of carbon dioxide a reconstitution of chlorophyll was possible from the products of its decomposition. What really happened was that the zinc salt of phaeophytin was formed, which closely resembles chlorophyll, and is easily mistaken for it. This zinc salt appears to be more resistant to acids and to photo-oxidation, and to be more stable than natural chlorophyll.

It is worthy of note that impure chlorophyll films prepared directly from the first petrol ether separation of an alcoholic

extract of chlorophyll always bleach more rapidly in light than do pure preparations of chlorophyll. No evidence could be obtained of the existence of any special oxidase enzymes in the impure films, but these always yield small amounts of reducing hexose sugar, even if previously well washed with water prior to photo-oxidation, whereas fully purified chlorophyll yields no sugar on photo-oxidation. In one test experiment, a sample of pure chlorophyll was prepared as described by Willstatter and Hüg (*Annalen*, 1911, p. 177). Two films of it were prepared each in a large bulb tube, containing dry CO_2 free air. After a week's exposure to sunlight, no further bleaching took place. The volume of air decreased by 14.6%, a small amount of water appeared, but no reducing sugar. After shaking with water and filtering, the latter gave Riminis, Schryver's, Schiff test, and the Schiff and sulphuric acid tests for formaldehyde. The amount present was, however, distinctly less than when a current of air was passed over long tubular films exposed to sunlight, and then led into water, as described in the previous paper.

The changes which chlorophyll undergoes during photo-oxidation in dead tissues are not necessarily precisely similar to those taking place after its extraction and purification. It has, however already been shown (*Proc. Royal Society B.*, 1908, 80, 30) that formaldehyde is produced in green leaves by the photo-oxidation of chlorophyll after death, and in the absence of carbon dioxide.¹ Green grass leaves were boiled and then dried in a strong press repeatedly until they yielded no trace of reducing sugar. The leaves were then spread flat on glass plates, and held in position by silk threads. The plates were placed in large glass vessels—(a) containing CO_2 free air, (b) with CO_2 present, (c) ordinary air. The vessels (a) and (b) were exposed to sunlight until the leaves were bleached, (c) was kept in darkness. The air in all three was kept moist. The leaves were then extracted with boiling water, which was concentrated, filtered and tested. Both (a) and (b) yielded small amounts of reducing hexose sugar, while (c) yielded none. Apparently the post mortem photo-oxidation of chlorophyll and its companion substances can increase the extractable sugar content of drying hay in the same way that photo-oxidation causes sugar to separate from an impure chlorophyll film.

¹ Further confirmation of this has recently been given by Ostertouit, see *n. ser.* 42, 1915, p. 63. See also Schryver, *Proc. Roy. Soc. London, B.*, 1910, 32, 226.

The influence of temperature.

Thin, bright green chlorophyll films formed on the floor of a beaker kept on a boiling water bath rapidly turned bright yellow. After twenty-four hours in darkness on the water bath, the films were nearly colourless. Comparative tests showed that although the bleaching of chlorophyll by oxidation at 100°C . is rapid, it is not as rapid as in sunlight at $30\text{--}35^{\circ}\text{C}$.

Films prepared from a petrol ether solution separated from an alcoholic solution, to which copper sulphate had been added, remained green after an hour on the boiling water bath, and still showed colour after twenty-four hours. The copper compound with chlorophyll is therefore more resistant than chlorophyll, not only to photo-oxidation, but also to thermo-oxidation.

Chlorophyll films in large sealed tubes filled with carbon dioxide kept at 100°C ., within one to two hours became bright yellow. The petrol ether extract was dark yellowish in colour, showed a strong fluorescence, and gave the spectrum of phaeophytin. It yielded no appreciable ash. Apparently at high temperatures carbon dioxide acts readily on chlorophyll, removing its magnesium and producing phaeophytin.

The photo-oxidation of xanthophyll.

In a previous paper it was suggested that portion of the oxygen produced during the interaction of carbon dioxide with chlorophyll might be used to oxidise xanthophyll into phytol, hexose sugars and formaldehyde. This suggestion was based upon data obtained by passing moist oxygen over dry "xanthophyll" films in tubes exposed to light. Further experiments carried out with films exposed to light under water kept saturated with air gave no trace of formaldehyde on distilling the oxidised liquid. The method of obtaining the xanthophyll used in these experiments yields a product which though free from chlorophyll, contains a high proportion of xanthophylloid and other impurities.

Owing to their presence, the impure "xanthophyll" obtained as previously described is temporarily soluble in petrol ether. Films from petrol ether solutions were covered with water exposed to sunlight and frequently shaken with fresh air, until the film breaks up to form a white, milky liquid. In addition strong alcoholic solutions were poured into water and similarly exposed. The products in this case are the same, but the bleaching is more rapid.

The watery liquid was then evaporated to dryness and the brown, gummy residue digested with a small quantity of cold water and filtered. The clear brown filtrate has a slightly sweetish taste, followed by the bitter flavour of wax impurities. If the digestion is prolonged in a larger quantity of water, the whole residue breaks up, and the less soluble portion swells to form a colloidal solid which is difficult to separate by filtering. It can, however, be condensed by the addition of a few drops of lead acetate.

The filtrate and also the filtrate from the photo-oxidation in water of impure chlorophyll from the first petrol ether separation gave the following tests:—

1. With α naphthol and sulphuric acid it gave the furfural test for carbohydrates (green ring, red above, purple on cooling and shaking).
2. Warmed with phloroglucin and hydrochloric acid, the solution deepened slightly in colour, but gave no pink. Hence no pentoses were present.
3. Strong reduction with Fehling's test not increased by previous warming with a drop of sulphuric acid. Hence no cane sugar present, but maltose, glucose or levulose.
4. With hydrochloric acid and resorcin it gave one warming a red liquid and a brown precipitate on standing. Since cane sugar is not present this shows the presence of a keto hexose such as levulose.
5. Milk of lime was added, and the liquid filtered.

Precipitate.—A portion dissolved in HCl. gave a fairly strong reduction with Fehling's test. A second portion was neutralised, and phenylhydrazin hydrochloride and sodium acetate added. After warming half an hour on a water bath a finely granular yellow glucosazone precipitate was given = presence of levulose.

Filtrate gave a blood red with picric acid and sodium hydrate.

Carbon dioxide was passed into the remainder of the filtrate, which was then evaporated to dryness, water added and refiltered. The filtrate gave a fairly strong reduction with Fehling's test, but not as strong as before the precipitation of the levulose. Presence of glucose.

In a further experiment, the watery extract from oxidised "xanthophyll," after clearing and filtering, was treated with ammoniacal lead acetate and filtered. The precipitate was washed, suspended in water, carbon dioxide passed through, and again filtered. The filtrate was evaporated to dryness, dissolved in a minimum of water, and cleared and decolorized with animal charcoal. The

solution had a sweetish taste, was dextro-rotary, and gave a strong reduction with Fehling's test.

The precipitate from the carbon dioxide was suspended in water, sulphuretted hydrogen passed through and filtered. The filtrate was evaporated to dryness, and the nearly colourless gummy residue dissolved in water and filtered. It was sweetish in taste, laevorotary, and gave a strong reduction with Fehling's test.

Hence the sugars produced are a mixture of dextrose and levulose, and probably in equal proportions since the original solution showed a slight laevorotation.

In a further experiment Pasteur's solution was added to a watery extract from photo-oxidised "xanthophyll," and a drop of water containing 10 to 20 yeast cells. The tubes were sealed, and kept at 25°C. In one week, yeast cells were abundant, and actively budding, carbon dioxide was formed, and the filtered liquid gave the iodoform test for alcohol. The sugars are, therefore, fermentable by yeast.

In the above experiments the "xanthophyll" used contained not only "xanthophylls," but also other extractives. Accordingly pure preparations of xanthophyll were prepared by slight modifications of the methods used by Willstätter and Mieg. (*Lieb. Ann.* 355 .1. 1917). Chopped dry grass leaves (4 kilogrammes) were extracted with alcoholic potash. The filtered liquid was treated with excess of ether and a separation effected by the addition of water. The red ether extract was washed with water, dried with anhydrous sodium sulphate, concentrated, and petrol ether added. The red precipitate which forms, was dissolved in hot acetone and filtered after cooling and standing, leaving a white solid, and a dark almost black liquid (orange red when dilute). To this, twice its volume of methyl alcohol was added, and on standing in darkness and out of contact with air, pure xanthophyll crystallizes out, and can be finally washed with petrol ether.

The xanthophyll obtained was exposed to photo-oxidation as dry films in tubes in a current of air, and in the form of a fine emulsion in water. No trace of reducing or non-reducing sugar could be detected after oxidation whatever the method used. In the first experiments traces of formaldehyde seemed to appear, particularly when the photo-oxidation was rapid. It was found, however, that the methyl alcohol used for precipitating the xanthophyll yielded traces of formaldehyde when exposed to sunlight. On removing this source of error no formaldehyde could be detected as a product of the oxidation of xanthophyll. It is worthy of note that pure

samples of xanthophyll oxidize and bleach much more slowly in light than do impure samples, xanthophyll being less readily oxidizable than chlorophyll, and very much less so than carotin. The white waxy solid separated by acetone during purification was exposed to light. No production of sugar or formaldehyde could be detected.

Dry films obtained from the first ether separation yielded small amounts of reducing sugar after photo-oxidation, and this even when the films had been previously well washed with water. The inference might be made that xanthophyll oxidized in the presence of certain associated substances yields sugar, but not when oxidized by itself. The addition of a few particles of magnesium powder retards the oxidation of pure xanthophyll greatly, but no sugar or formaldehyde appears.

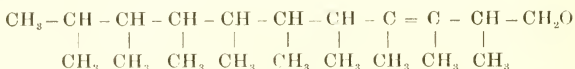
To an alcoholic solution of impure "xanthophyll" which yielded sugar readily on photo-oxidation, alcoholic potash was added. A reddish yellow precipitate formed, insoluble in petrol ether, acetone or ether, but dissolving readily in water, and consisting almost wholly of reducing sugar. On now separating the xanthophyll solution with ether, no trace of sugar could be obtained from dry xanthophyll films after photo-oxidation. The impure xanthophyll films evidently contain preformed sugar, possibly held in loose combination, so that it is not readily removed by washing with water, but is easily separated from an alcoholic solution by the addition of potash.

In the purification of the ether solution of xanthophyll by precipitation with ether, a large amount of yellow xanthophylloid material remains in solution, and is not precipitated from an acetone solution by methyl alcohol. This forms a yellow oily solid with a low melting point. It is readily soluble in petrol ether. Boiled with water it yields neither formaldehyde nor reducing sugar. Films exposed to sunlight bleach fairly rapidly. They usually yield a trace of acetone (Schiff test, etc.) when previously washed and dried, but give no formaldehyde, Schryvers, Rimmers, and the Schiff, and sulphuric acid tests being all negative. The watery extract from the bleached films when evaporated to dryness dissolved in a little water and filtered, gave in all cases the test for a reducing hexose sugar.

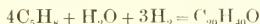
In a further test, with pure xanthophyll, nearly half a gram was spread as a film inside a large flask exposed to strong sunlight, with a slow current of moist CO_2 -free air passing through the flask and then into water in a darkened receiver. The film was wholly

bleached in three days. The water in the receiver gave Schiff's test strongly, Schryvers, Rimmers, and the Schiff and sulphuric acid tests faintly. It apparently contained a trace of formaldehyde in addition to a trace of acetone, but not sufficient of the former to give the characteristic smell.

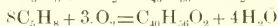
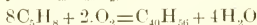
Apparently when subject to violent photo-chemical oxidation pure xanthophyll may produce traces of formaldehyde, but none when the oxidation takes place in darkness or in weak illumination or under water. According to Willstätter, Mayer, and Hüni (Annalen, 1910, p. 73), the constitutional formula of phytol may be



Phytol would therefore be constructed from similar building materials to aliphatic and cyclic terpenes and rubber. Just as geraniol and limonol may be constructed from two molecules of isoprene, so may four molecules form phytol, as thus:—



In the same way eight molecules of isoprene might yield carotin ($\text{C}_{40}\text{H}_{56}$) or xanthophyll ($\text{C}_{40}\text{H}_{56}\text{O}_2$).



It would not be surprising that under violent photo-oxidation some of the $\text{CH}-\text{CH}_3$ components of carotin, as well as of phytol, and hence also of chlorophyll, might be torn away, and oxidized to formaldehyde, and as thus $(\text{CH}-\text{CH}_3) + \text{O}_2 = 2\text{CH}_2\text{O}$. If so, the presence of two additional atoms of oxygen in the xanthophyll molecule renders it not only more stable in this respect, but also more resistant to oxidation than is carotin.

According to Willstätter and Stoll, pulverised xanthophyll takes up 36.5% by weight of oxygen and produces a compound which when precipitated from methyl alcohol by ether has the formula $\text{C}_{40}\text{H}_{56}\text{O}_{18}$. This is, however, merely a percentage formula, and gives no indication of the mode of union of the oxygen.

The oxidation of carotin.

In the experiments described in the previous paper, films of carotin were exposed to light in a current of moist air. Using watery emulsions of carotin exposed to light and air, the oxidation was much more rapid than in the case of xanthophyll, the liquid

when distilled yielded appreciable quantities of formaldehyde, but no trace of reducing sugar was formed.

The quantitative weighings of dry films during oxidation indicated that further oxidization is possible after the carotin has been bleached white.

The presence of an oxidase strongly accelerates the oxidation of carotin both in light and in darkness. A solution of carotin in hot alcohol was poured into water so as to form a yellow turbid emulsion. This was divided into four parts, A, B, C, D. To A and C a few drops of a solution of copper sulphate and salt were added, which acts as a strong oxidase. A and B were exposed to sunlight, C and D were kept in darkness in beakers with a large free surface exposed to air. In A and C the emulsion, owing to some mechanical action of the copper sulphate, aggregates to larger particles, which would tend to delay oxidation, but nevertheless A was fully bleached in two days, B in four days, C in six days, and D even after eight days was still reddish yellow, but somewhat paler. A yielded a distinct trace, B a doubtful trace, and C and D no trace of formaldehyde on distilling.

A curious result was given by a carotin solution obtained by boiling the expressed juice of grated carrots, washing the floating red mass with absolute alcohol, dissolving in hot absolute alcohol and separating with petrol ether. Emulsions were made by evaporating to dryness, dissolving in absolute alcohol, and adding water. Repeating as above, the oxidized liquids were filtered, evaporated to dryness, and the residue digested with cold water for twenty-four hours, filtered, and then tested for reducing hexose sugars. A gave strong positive results, B weak positive results, C fairly strong positive tests, and D doubtful or negative tests.

The appearance was given as though the addition of a metallic oxidase caused carotin to oxidize to carbohydrates. The solutions used, however, contained carotinoids as well as carotin, and carotin purified by precipitation did not yield any sugar on oxidation under any conditions.

From the original alcoholic carotinoid solution sugar separated, when it was treated with alcoholic potash. The liquid A and C after oxidation filtered clear, whereas B and D filtered turbid, and left waxy residues, which apparently held sugar as a physical mixture or in loose chemical union, and from which even long contact with cold water removed the sugar only to a slight extent or not at all. Hence the apparent production of sugar on photo-oxidation in the presence of copper sulphate and salt.

The conversion of xanthophyll into carotin.

Haas¹ and Hill state apparently on the authority of Palladin² that carotin is converted by a plant oxidase into xanthophyll, and xanthophyll by a reductase into carotin. The same statement is definitely referred to Palladin by C. J. West,³ but is evidently transcribed from Haas and Hill. As a matter of fact Palladin does not mention either carotin or xanthophyll in the papers quoted. He shows that reductases are present in plants as well as oxidases. The respiratory oxidases, he considers to be pigment forming oxidases, and (on p. 385) "at present we only know that the respiratory chromogens are aromatic compounds; while in the living plant the respiratory pigments usually occur as colourless chromogens, and when present in coloured form (beet root, etc.) can be reduced to colourless compounds."⁴

In my previous paper⁵ I was also misled into ascribing this statement to Palladin, but could obtain no evidence of any action of either watery or glycerine extracts of plants oxidases and reductases on carotin or xanthophyll. This may possibly be because of the difficulty of reproducing the conditions existing in the plant. Experiments were, however, made with the pigments in various solvents, and in the form of fine emulsions in water.

The results obtained with metallic reductases were more satisfactory. As already shown, magnesium dust rapidly, and zinc dust slowly, reduce xanthophyll, present in a clear yellow solution in a mixture of alcohol and water, to carotin, leaving the liquid nearly colourless. Using strong emulsions of xanthophyll in the form of suspended particles, zinc dust was found to be ineffective and magnesium dust only acted slowly. In the latter case on filtering, drying and dissolving, the dry residue in petrol ether, the bulk of the pigment consisted on evaporating of yellow xanthophylloids with scattered minute red particles of carotin. The tests should be done in darkness, and as far as possible out of contact with the air since otherwise the carotin may oxidize to colourless products.

All attempts to convert carotin into xanthophyll by the aid of metallic oxidases, organic oxidases (carrot, potato, apple) or direct

1 Chemistry of Plant Products, p. 252.

2 Ber. d. D. Bot. Ges. 1908, 26, A, pp. 125, 378, 398; (1909), 27, p. 110. Zeitschr. f. Physiol. Chemie. (1908), 55, p. 207.

3 Biochemical Bulletin, 1915, p. 184.

4 Zeits. f. Physiol. Chem., B4. 55, 1908, p. 221.

5 Proc. Roy. Soc., B, 89, 1915, p. 11.

oxidizing agents failed, whether solutions or emulsions in water were used. Lubimenko³ finds, however, that both carotinoids and xanthophylls exist which are intermediate between xanthophyll and carotin. The carotinoid from the aril of *Euonymus* is indeed insoluble in petrol ether, while xanthophylls are soluble to a greater or lesser extent. According to Lubimenko, in the absence of oxygen, the xanthophyll decreases and the carotin increases, while in boiled leaves exposed to air and peroxidase, the carotin is converted into a yellow pigment equally soluble in alcohol and petrol ether, and with a spectrum intermediate between carotin and xanthophyll.

The influence of the absence and presence of carbon dioxide.

Mustard seedlings were grown in moist air in diffuse daylight, and under similar conditions in air deprived of carbon dioxide. The cotyledons in the first case were larger, broader and more yellowish green, and in the second were smaller, darker and more bluish green. The cotyledons with the upper half of the hypocotyl were removed, and the crushed material after killing by boiling was extracted with successive portions of absolute alcohol, and finally with hot alcohol. The last extraction was nearly pure carotin, and showed clearly that the seedlings grown in the absence of carbon dioxide contained more carotin than the others.

The whole extract was divided into three parts. From one the chlorophyll was separated by three successive petrol ether extractions, and the amount of chlorophyll estimated by comparison with solutions of known strength. The second sample was evaporated to dryness, dissolved in a minimum of cold absolute alcohol, saponified and separated with ether. The third sample was saponified, petrol ether added, and then water. After washing the petrol ether and ether solutions, their strengths were estimated by comparison with solutions of known concentration. This method is not wholly accurate, but in the complete separation of chlorophyll, carotin and xanthophyll, the losses are so great that the exact estimation of the amounts originally present is impossible.

The values obtained were:—

		Weight of Material. grammes.		Chlorophyll. grammes.		Carotin. grammes.		Xanthophyll. grammes.
CO ₂ present	-	100	-	0.474	-	0.11	-	0.15
CO ₂ absent	-	100	-	0.271	-	0.275	-	0.12

¹ Compt. Rend. 1915, p. 278.

The dark blue green colour, therefore, appears to be due not to an excess of chlorophyll, but rather to the more compact character of the tissue, and it appears as though chlorophyll develops most rapidly when its normal functional activity can be exercised. The conclusion seems also justified that the carotin supplied at least a part of the carbon and hydrogen for the construction of chlorophyll.

The spontaneous decomposition of chlorophyll.

Twenty pounds of grass leaves were extracted with absolute alcohol, and separated with two litres of petrol ether. After washing with 80% alcohol, the petrol ether solution containing a little alcohol and a trace of water was placed in a completely filled bottle. The percentage of chlorophyll was estimated by diluting against a sample of pure chlorophyll and comparing under the spectroscope. The bottle was sealed and kept in darkness for one year. On opening and diluting a sample the colour was strongly yellowish green instead of pure green. The solution was divided into four parts.

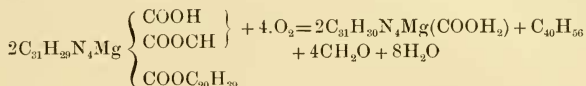
One part was evaporated to dryness, and treated with cold absolute alcohol. This left behind a large amount of a brownish white waxy film, readily soluble in petrol ether. The solution was diluted down to a dilute solution of known strength, in which only the red band of chlorophyll was visible. This indicated that per gram of the original chlorophyll only 0.54 gram remained.

To the second portion of the petrol ether solution twice its volume of absolute alcohol was added, and sufficient hot water to bring about separation. After standing three days in darkness the supernatant liquid was deep yellow, and a layer of solid red particles was present between the two liquids. These were collected and washed. They consisted mainly of xanthophyll, with a little carotin.

The third portion was evaporated to dryness in the form of a film lining a large tube. It weighed 4.452 gram. After soaking in cold absolute alcohol for several days, the brown waxy film remaining was washed and dissolved in petrol ether. On evaporating, it weighed 1.309 gram, and on incinerating after warming with a drop or two of nitric acid it yielded 6.8 per cent. of ash as magnesium oxide. According to Willstätter, the phyllins (glauco-phyllin and rhodophyllin have the formula $(C_{31}H_{30}N_4Mg(COOH_2))$ which represents 7.1% of ash as magnesium oxide.

The fourth portion was agitated with a little water, the petrol ether allowed to evaporate and the residue distilled. The distillate contained distinct traces of formaldehyde.

Hence impure chlorophyll appears to spontaneously undergo partial segregation into carotin and xanthophyll, and pale brown waxy solids rich in magnesium. In addition small quantities of formaldehyde appear. An equation for the production of glaucophyllin and carotin from chlorophyll cannot be made to balance without the addition of oxygen, but small quantities of dissolved or occluded oxygen might have been present before sealing, as this possibility was overlooked. In that case a suggested equation might be—



If a similar change resulted in the production of the monocarboxylic phyllophyllin ($C_{31}H_{31}N_4Mg.COOH$) still less oxygen would be required.

In samples of chlorophyll purified by precipitation, no carotin or xanthophyll appeared, and dry samples kept in sealed vacuum tubes were apparently unaltered after five months in darkness. In the case of dry samples from the unpurified petrol ether extract, after keeping for some months, the sample will flow slowly over the glass, and shows a separation of solid red carotin or xanthophyll particles.

The Precursors of Chlorophyll.

According to Timiriazeff¹ and to Monteverde² etiolated leaves yield a small amount of fluorescent pigment which they term "proto-chlorophyll." This, according to Monteverde and Lubimenko,³ is a derivative of the labile "chlorophylligen," which rapidly turns into chlorophyll when exposed to light. The precursor of this theoretical substance is, according to the same authors, a colourless theoretical substance, "leucophyll."

Kohl⁴ showed that etiolated seedlings contained carotin in abundance, and even doubted whether any other pigment was present. He also found that the percentage of carotin did not decrease during the formation of chlorophyll on exposure to light, and hence denied that any conversion of carotin into chlorophyll components took place. Kohl's observation might, however, merely indicate that while food materials were abundant, the production

¹ Compt. rend., 102, 686 (1886); 414 (1889).

² Acta. Horti. Petropol., 13, 201 (1894); Bull. Lard. Imp. Bot. Petrograd, 7, 37 (1907).

³ Biol. Central, 31, 449 (1911); Bull. Ac. Imp. Sci. Petrograd, 73 (1911); 609 (1912).

⁴ Untersuchungen über Carotin, 75 (1902).

of carotin continued at a greater rate than it was used in the formation of chlorophyll.

To test this, two batches of wheat seedlings were grown in darkness, until approaching partial starvation. One batch was then exposed to full sunlight for one day. Equal quantities were then dipped in boiling water, the surplus water squeezed out, and the leaves twice digested with cold absolute alcohol on two successive days. The fully etiolated leaves were still deep yellow, and with hot alcohol gave a strong, nearly pure solution of carotin. Those which had been exposed to light were nearly colourless, and gave a pale yellow solution with hot alcohol, containing only a small amount of carotin.

Kohl is, however, correct in stating that carotin is the chief pigment in etiolated seedlings. Complete extractions with etiolated wheat seedlings yielded from 8 to 10 parts of carotin to one of xanthophyll, the percentage may of course vary with other seedlings, and after prolonged etiolation it appears to decrease.

If colourless precursors of chlorophyll exist, they should have a high magnesium content, and should be capable of separate extraction. An area of ten square feet of closely planted wheat was kept in darkness until the seedlings were six inches high. The yellow leaves were cut at once and dropped into boiling water, and after squeezing out all excess of water, twice digested in cold alcohol for periods of a day, all liquid being squeezed out in a strong press after each extraction. The nearly colourless material was then digested in boiling absolute alcohol for five hours. The solution was filtered hot, petrol ether added while still warm, and sufficient water to bring about separation. After standing for a day the pale yellow petrol ether extract was evaporated to dryness, and the residue dissolved in a minimum of hot alcohol. In this solution, after standing for one day, a bulky white crystalline solid separates out which appears to contain alcohol of crystallization. After filtering and washing with a little absolute alcohol a pale brownish white solid was obtained. This gave off alcohol when heated, and after incinerating with a drop of nitric acid yielded 1.5% of ash as magnesium oxide.

The original precipitate, however, appears to consist of two solids—(a) a white wax less soluble in cold alcohol than carotin, but readily soluble in hot alcohol; and (b) a browner wax, which is still less soluble in alcohol, than the preceding, and can be separated from it by washing with small amounts of warm alcohol. This less soluble browner wax yielded in one experiment 6.2% of ash as mag-

nesium oxide, and in another 7.4%. The discrepancy is considerable, but the difficulty of purifying without great loss of material is very great. The amounts of solid obtained for incineration were only 0.35 and 0.42 gram. respectively. Presumably, the original precipitate consists of a darker waxy solid, with a high magnesium content, and a paler wax with little or no magnesium, which may possibly partly be derived from the wax impregnating the cuticle. and have nothing to do with chlorophyll.

The wax present in the cuticle does not, however, decrease with starvation, and increases in amount with increasing age. In a further experiment the seedlings were grown in darkness until nearly starved, and the yellow leaves were treated as before. The waxy solids obtained after separation from petrol ether and crystallization from hot alcohol gave a yield of 5.1% magnesium oxide. The amount obtained was insufficient for the separation into magnesium containing and non-magnesium containing waxes, but the result indicates that the waxy solids with little or no magnesium decrease during starvation, and hence are not derived wholly from the cuticle.

The decomposition of chlorophyll in grass leaves in darkness.

Plots of barley grass (*Hordeum murinum*) were kept in darkness from just before the commencement of flowering until the greater part of the leaves turned yellow or yellowish red. These parts were cut away and extracted. A little chlorophyll was still present, as well as xanthophyll and carotin, in the approximate proportions of 1, 3 and 12 respectively. The pale brownish white wax obtained as previously described yielded 1.15% of ash. After washing with warm alcohol the darker residue remaining yielded as much as 12.9% of ash, but the whole of this did not consist of magnesium oxide.

Many of the old grass leaves were bright red in colour, and remain so in darkness, until they died and shrivelled. These were extracted with absolute alcohol and petrol ether until all carotin and xanthophyll were removed. They were still red. They imparted a red tinge to water and the red pigment rapidly dissolved in dilute hydrochloric acid or dilute potash. The neutral solution in water was precipitated by lead acetate, turned yellow with sodium hydrate, reddish brown with hydrochloric acid, and dark reddish brown, with ferric chloride. It was apparently, therefore, a flavone, and as it appeared to develop in or in the neighbourhood of the chloroplastids, it may be one of the products

of the progressive decomposition of chlorophyll in darkness. In any case the formation of a pigment of this character is darkened leaves in unusual. Extractions of additional material, with alcoholic potash containing a little water yielded potassium rhodophyllin, but whether this was partly responsible for the red colour or was merely derived from the chlorophyll, is impossible to say.

In the case of most green leaves, the chlorophyll decomposes more or less rapidly in darkness. This is possibly primarily due to the action of the carbon dioxide produced by respiration, aided by subsequent oxidation, and by the absence of the chlorophyll regeneration normally carried on in the presence of light. It is worthy of note that in fleshy plants such as cacti, where in darkness a formation of organic acids may largely replace the normal carbon dioxide production, the leaves remain green unusually long in darkness. The same applies to water plants such as *Elodea* and *Chara*, in which the carbon dioxide is removed in solution by the water outside.

The photo-oxidation of rhodophyllin.

An alcoholic solution of chlorophyll containing a little water was heated on a water bath with potash for twelve hours. Excess of water was added, and the liquid was placed in a stoppered Winchester, shaken and allowed to settle daily for three days. The supernatant liquid was syphoned off, and the residue filtered. The residue was well washed with water, dried washed with a little absolute alcohol, and then treated with hot alcohol containing a little water. The red filtrate on standing formed red crystalline platelets, with a bluish metallic lustre. From this potassium rhodophyllin, rhodophyllin was obtained by treating with acid sodium phosphate and subsequent separation.¹ The product was readily soluble in ether and alcohol.

Films and watery emulsions of rhodophyllin and of its potassium salt were exposed to light. The latter was slightly more resistant to photo-oxidation than the former, but both proved to be much more stable and resistant to photo-oxidation than either chlorophyll or xanthophyll. No formaldehyde or reducing sugar could be detected as a product of the photo-oxidation, either in the absence or in the presence of carbon dioxide.

Since rhodophyllin is comparatively stable, and resistant to photo-oxidation, it would be natural to find that it would be one of the products of the decomposition of chlorophyll in autumnal

¹ Willstätter and Pfannestül, *Annalen*, 358, 1908, v. 205.

leaves exposed to light. Brown leaves of *Salix Babylonica* were collected in quantity and boiled and pressed repeatedly until all the soluble tannins were removed. They were then washed with absolute alcohol, and extracted with hot absolute alcohol.

The liquid was filtered, concentrated and cooled and refiltered. The brown waxy solid removed consisted partly of phaeophytin. Ether was added to the alcohol and then salt. The reddish yellow ethereal layer contained all the pigment. After washing with water and drying with sodium sulphate, it was evaporated. The residue was extracted with methyl alcohol, leaving a white waxy solid undissolved. To the liquid, alcoholic potash was added, and after heating, it was filtered. On standing, shining red platelets with a steely blue shimmer of potassium rhodophyllin separated out.

Yellow leaves picked off the tree and kept in darkness for three days turned brown, as in fallen leaves, so that the change is not necessarily due to light. They yielded, however, xanthophyll largely on extraction and rhodophyllin only in small amount.

Rhodophyllin was also obtained from the brown autumnal foliage of the English oak. When these are red in colour erythrophyll is present in the cell sap, but it soon fades, leaving them a pure brown colour. The colour of such leaves is due to several pigments of which the erythrophyll fades first, then the xanthophyll, then the rhodophyllin and the permanent brown colour is due practically wholly to oxidized tannin compounds.

The evolution of oxygen from etiolated plants.

It appears from the foregoing that etiolated leaves contain carotin, a little xanthophyll, and a nearly colourless waxy solid rich in magnesium, which is either glaucophyllin, or is related to it. Carotin appears to be the first pigment formed in the construction of chlorophyll, and presumably it is produced at the expense of carbo-hydrate or of hydrocarbon food materials. The relative amounts of carotin and xanthophyll in etiolated parts may depend upon the relative activities of oxidase and reductase enzymes respectively.

When an etiolated plant turns green in light, it seems reasonable to suggest that the carotin undergoes photo-oxidation,¹ liberating formaldehyde, which is rapidly polymerized, and that the bleached

¹ In the case of plants able to develop chlorophyll in darkness, an oxidase enzyme could produce the same effect.

carotin residue combines with the glaucophyllin, converting it into the tricarboxylic chlorophyll. This process involves an absorption of oxygen. If carbon dioxide is present, it interacts with the chlorophyll, probably undergoing additive combination. If it then separated formaldehyde and phytol, it would liberate a large amount of oxygen. The phytol would then recombine with the glaucophyllin, reforming the chlorophyll molecule. If the supply of carbon dioxide was abundant in proportion to the intensity of illumination, the decomposition and recombination would balance and an evolution of oxygen would be possible without any actual accumulation of chlorophyll taking place. In 1896 I was able to confirm on a variety of plants the statements of Draper and of Englemann that etiolated chloroplastids can evolve oxygen in light,¹ and showed that this took place before any actual chlorophyll was developed. The rule was not a universal one, however, and particularly in the case of maize, which has a high temperature minimum for the formation of chlorophyll, no evolution of oxygen could be detected from etiolated leaves so long as they were free from chlorophyll. It was also shown that sealed preparation of etiolated leaves of *Elodea* with Bacteria, owing to the presence of an excess of carbon dioxide did not turn green in light, although they produced the small amount of oxygen necessary to support protoplasmic streaming.

The presence of from 2% to 5% of carbon dioxide in the surrounding air distinctly retards the turning green of etiolated grass seedlings as compared with those in ordinary air,² but if the intensity of the illumination is approximately quadrupled, the retarding action of the carbon dioxide is less pronounced or even ceases to be perceptible. Kohl³ ten years later without referring to the previous work reconfirmed the statement that etiolated plants may evolve oxygen in light. He showed that carotin was the chief pigment in etiolated plants, and held that it was responsible for the assimilation of carbon dioxide in the absence of chlorophyll. It is, however, more probable that the evolution of oxygen takes place as outlined above, and that carotin is not by itself capable of causing the assimilation of carbon dioxide. Actual observation shows that it does not appear to combine with carbon dioxide, and that it combines more energetically and rapidly with oxygen than either chlorophyll or xanthophyll does. The formaldehyde produced

1 See Journal of Linnean Society, vol. xxxi., 1896, p. 554.

2 See also Bohm, Sitzungsab. d. Wien. Akad., 1873, p. 14.

3 F. G. Kohl, Ber. d. D. Bot. Ges., 1906, vol. xxiv., p. 222.

during its photo-oxidation does not appear to be directly derived from carbon dioxide.

Kohl states that etiolated plants exposed to light in the absence of oxygen, but in the presence of a little carbon dioxide, slowly turn green, the oxygen set free by the agency of the carotin being used in the formation of chlorophyll. It is doubtful, however, whether a complete absence of oxygen was assured at the outset, for Correns¹ has shown that the production of chlorophyll is closely dependent upon the presence of oxygen, and that a partial pressure of oxygen, at which growth and heliotropic curvature are still possible, does not suffice for the formation chlorophyll.

The evolution of oxygen from green plants in light.

It is a difficult problem to determine how the oxygen produced in close contact with pigments capable of rapid photo-oxidation is able to escape from the cell. No production of oxygen could be obtained from extracted chlorophyll, carotin or xanthophyll in the presence of oxidase or reductase enzymes in light or darkness and in the presence or absence of carbon dioxide. It seems legitimate, however, to assume that the large chlorophyll molecules may have a definite physical arrangement in the protoplasm of the chloroplastids. If their phytyl radicles were all turned outwards towards the entering carbon dioxide, and carotin or xanthophyll were produced by additive combination with the latter, the surplus oxygen could either escape or disintegrate the carotin or xanthophyll, back to phytyl. For the latter a portion only of the oxygen is required. The oxidation films formed from carotin, chlorophyll and xanthophyll are very impermeable to oxygen, so that the excess would diffuse outwards. So long as the supply of carbon dioxide was sufficient to replace the xanthophyll or carotin film, as it was oxidized, and as the phytyl returned to the chlorophyll molecule, the chlorophyll would remain unoxidized, but if the intensity of illumination increased greatly, oxygen would slowly penetrate the chloroplastid, bleaching it.

Phytol itself forms a series of compounds with varying proportions of oxygen ($C_{20}H_{38}O_2$, $C_{20}H_{34}O_3$), etc.,² and it also forms an ozonide $C_{20}H_{40}O_3$, which spontaneously separates into $C_{20}H_4O$, O_3 and free oxygen.³

The possibility of a reductase enzyme, converting zanthophyll into carotin, with a liberation of free oxygen also needs considera-

1 Correns, *Flora*, 1893, p. 14.

2 Willstatter and Hocheder, *Annalen der Chemie*, 1907, 353, p. 205.

3 Willstatter, Mayer and Huni, *Annalen*, 1910, p. 73.

tion. Against this possibility we have the fact that free oxygen oxidizes carotin more rapidly than xanthophyll, and that the only reductases which seem able to effect the reduction of xanthophyll to carotin appear to be such as themselves readily combine with free oxygen.

In the absence of light the presence of a film of carotin or xanthophyll on the surface of the chloroplastid would render the further penetration of carbon dioxide very slow, and would largely protect the chlorophyll, so long as the plastid was living, from decomposition by carbon dioxide during darkness.

The now well recognised fact that chloroplastids may be rendered temporarily inactive without necessarily being killed, and while appearing normal,¹ is sufficient evidence that the continued assimilation of carbon-dioxide involves a definite relationship between the chlorophyll and protoplasm of the chloroplastid. In other words the physical structure of the chloroplastid may be as important as the chemical composition of chlorophyll. Some special arrangement must exist to protect the chlorophyll from the direct chemical action of the carbon dioxide, which would otherwise remove the magnesium from the chlorophyll and convert it into phaeophytin.

The polymerization of formaldehyde.

Butlerow (Liebig's Annalen, 120, p. 295, 1861) obtained a bitter tasting syrup "methylenitan," by the action of lime water on trioxymethylene, a polymer of formaldehyde. Loew obtained a sweet syrup by the prolonged action of lime water on 4% formaldehyde. Euler has shown that when a 2% solution of formaldehyde is heated for some hours with calcium carbonate, arabinoketose, a pentose sugar, is produced with glycollic aldehyde as an intermediate product. According to Czapek (Biochemie, 1913, Vol. 1, p. 628), glucose is not produced by the action of alkalies on formaldehyde, but only non-fermentable sugars such as i-Fructose² and i-arabinoketose. Fischer, however, obtained from Loew's crude formose an "acrose" sugar, which he was able to convert into levulose.

Both Loew's and Euler's methods are slow and tedious to carry out. A rapid method is as follows:—A saturated solution of formaldehyde is mixed with six times its volume of lime water, and

¹ Ewart, Journal Linnean Society, 1896, vol. xxxi., p. 364.

² Ordinary fructose is fermentable by yeast. See Harden and Young, Proc. Roy. Soc. London, B., 1910, 82, p. 645.

while kept heated to boiling point one and a-half volumes of 12% sodium hydrate are added. The latter portions are added slowly, and if necessary a few drops additional. A white precipitate is formed at first, with each addition of sodium hydrate, which redissolves slowly, the liquid remaining alkaline. With the addition of the last few drops, the liquid suddenly clears, become pale yellow, ceases to smell of formaldehyde, but develops a slight caramel smell, and is neutral. If an excess of sodium hydrate is added, the liquid turns brown, and a precipitate may form, but it may be neutralized with a drop or two of acid. On cooling and standing, a white crystalline solid separates, which is insoluble in absolute alcohol, and practically so in cold water. On heating it chars, glows and leaves a bulky chalk ash. It is, therefore, an organic calcium compound. The filtrate gives all the tests for a reducing hexose sugar. It also gives red with picric acid, and sodium hydrate, and gives a caramel smell, and darkens when warmed with sulphuric acid. It gives the keto-hexose reaction, with resorcin and HCl. With phenylhydrazin and sodium acetate, it gives a golden yellow imperfectly crystalline osazone. The liquid showed a feeble laevo rotation.

The diluted liquid, after adding yeast and Pasteur's Solution, keeping at 30°C. for three days, and distilling, gave a product containing an appreciable quantity of alcohol, and giving the iodoform test readily. The sugars are, therefore, in part at least fermentable.

The following procedure was adopted to separate the different products:—

The original syrup contains several compounds. After no more of the insoluble calcium compounds would crystallize out, the syrup was filtered, and twice its volume of absolute alcohol added. A colourless viscid liquid separates and falls to the bottom. After washing this with alcohol it becomes a white viscous solid. On adding water it partly dissolves and a white crystalline solid separates, which when heated chars with a slight caramel odour, and leaves an alkaline ash containing CaO and CaCO_3 . The filtrate, after the addition of water, gives a strong reduction with Fehling's test. On evaporating nearly to dryness, and cooling, a white crystalline solid separates, which is an organic sodium salt, and the syrup contains sugar. It yields a glucosazone, but gives only a faint reaction with resorcin and HCl., and appears to contain more glucose than levulose.

The original filtrate from the first addition of alcohol is a clear, slightly yellow liquid. On adding twice its volume of alcohol, it becomes turbid; after standing one day a bulky mass of long, slender crystalline needles separates. This, after washing with alcohol, gives no reduction with Fehling's, but chars on heating, and gives an ash of sodium carbonate. It is readily soluble in water, and consists of an organic sodium salt.

The yellow filtrate on evaporating to a thick syrup and cooling, crystallises out the remainder of the sodium salt. The filtered syrup contains mainly hexoses, but also some pentose sugar. Thus distilled with HCl. it turns aniline acetate strongly red, while with HCl. and phloroglucin it turns red, and then gives a brown precipitate soluble in amyl alcohol.

The purified syrup was treated with ammonical lead acetate, and filtered. The filtrate contained pentose sugar. It was evaporated to a small bulk, after treating with CO_2 and filtering, cooled, the liquid drained off, diluted, and Pasteur's ash and yeast added. No alcoholic fermentation took place.

The washed precipitate was treated with carbon dioxide and filtered. The filtrate was evaporated to dryness, dissolved in a little water and filtered. It gave the test for glucose, was dextrorotatory produced a glucosazone, and after adding yeast and Pasteur's ash, the liquid distilled after three days readily gave the iodoform reaction for alcohol.

The remaining solid was treated with sulphuretted hydrogen, and the filtrate evaporated to dryness and dissolved in water. It gave the ketose reaction, was laevorotatory, and produced glucosazone. After adding yeast and Pasteur's ash, the liquid distilled readily, and gave the iodoform reaction for alcohol. Both sugars were also capable of nourishing putrefactive bacteria, *Penicillium* and *Mucor*, when infected with these organisms.

The proportions given above are the most satisfactory for a complete reaction. With less lime water the reaction is less perfect, and less sugar is produced. With more lime water the boiling liquid clears only slowly, between each addition of sodium hydrate, and it is more difficult to obtain a perfectly neutral liquid at the end of the reaction. On cooling, however, a larger amount of the calcium salt crystallizes out directly, as for instance, when the proportions used are 50 c.c. of 40% formaldehyde, 500 c.c. of lime water, and 65 c.c. of 12% sodium hydrate. The crude sugar consisted almost entirely of hexoses, with apparently a trace of pentose, as it gave the furfural test more readily than a pure hexose solu-

tion, and gave a slight coloration and turbidity with phloroglucin and HCl.

A more rapid but less perfect separation than that given above is effected by evaporating the whole synthesized product nearly to dryness in a water bath, and adding a minimum of cold water. The calcium salt remains undissolved. The filtrate is again evaporated nearly to dryness, and treated with 90% alcohol. The residue consists of sodium tartrate; the filtrate contains the sugar.

In previous work on sugar synthesis, alkalies have been used singly instead of jointly, and not as here described. As the by-products described have been saccharates, formates, etc., with which the calcium and sodium salts did not agree, a precise determination of their character was necessary.

Professor E. W. Skeats investigated the crystallographic character of the calcium salt, and found that it belonged to the orthorhombic system, forming pyramids, domes and elongated pinacoid pyramids, with parallel sides. The crystals were strongly, doubly refractive, optically negative, and had a mean refractive index of 1.55. Mr. J. W. Clendinnen carried out a complete analysis of pure samples of the two salts.

Analysis of two Crystalline Salts obtained by Professor Ewart.

The calcium salt was in the form of well defined crystals, and so needed no further treatment to purify it. As the salt was evidently one of an organic acid, simple ignition left a residue of calcium oxide, from which the percentage of calcium could be calculated.

A combustion was then done with the calcium salt, and this gave the percentages of hydrogen and carbon, and thus oxygen by difference.

These were the results obtained:—

Element.		Percentage.			At wk.		Approx. Ratio At wk. \times .385.
		Duplicates.	Mean.				
Ca	-	15.37 } 15.45 }	- 15.4	-	.385	-	1
H	-	4.91 } 4.90 }	- 4.9	-	4.9	-	12
C	-	18.84 } 18.69 }	- 18.7	-	1.56	-	4
O	-		- 61.0 (diff.)	-	3.81	-	10

This formula ($\text{CaH}_{12}\text{C}_4\text{O}_{10}$) corresponds with that of calcium tartrate $\text{CaH}_4\text{C}_4\text{O}_6 \cdot 4\text{H}_2\text{O}$. The usual quantitative tests for tar-

trates were then applied to both the salts. All the tests were positive, thus confirming that these were salts of tartaric acid.

The importance of this method of producing sugar lies in the fact that it is a rapid and well defined action, yielding definite salts, namely, sodium and calcium tartrates, and that the amount of sugar produced, particularly of hexose sugars, is large, being intermediate between the weights of the calcium and sodium salts. It is not, however, likely that this mode of producing sugar from formaldehyde is of any importance in the living plant, since it involves a high temperature, and the presence of an abundance of two free alkalies.

Willstätter's and Stoll's¹ work on Photo-synthesis.

These authors found that all attempts to produce extracellular photo-synthesis failed, which they conclude is owing to the absence of a hypothetical enzyme from extracted chlorophyll. They found, however, that slight pressure applied to the leaf completely stopped photo-synthesis, which rather supports my own view than an orderly physical arrangement of the chlorophyll molecules in the chloroplastid is an essential factor in continuous photo-synthesis, and that a disturbance or disorganization of this arrangement may be partly responsible for the temporary inactivity into which an apparently normal chloroplastid may be thrown by various agencies or treatments, which when extreme, lead to death and permanent disorganization.

They also conducted experiments on living leaves by passing a mixture of air and carbon dioxide over them in vessels on a water bath exposed to light, and estimating the amounts of carbon dioxide assimilated. They found that the assimilation was not always proportional to the chlorophyll content, a fact already well known to the plant physiologist.

In leaves rich in chlorophyll the authors found that increased illumination did not increase the assimilation, whereas a rise of temperature did, while in leaves deficient in chlorophyll a rise of temperature had little effect, and increased illumination rendered the assimilation of carbon dioxide more active. Hence they conclude that their hypothetical enzyme is in relative excess, and only exercises its maximum effect when the chlorophyll is working at full pressure.

¹ Berichte, 1915, 48, 1540.

Willstätter and Stoll have, however, overlooked the principle of limiting factors established by Blackmann and his pupils. In leaves rich in chlorophyll the limiting factor is usually the supply of carbon dioxide and an increase of illumination beyond that necessary to assimilate the carbon dioxide available in a given unit of time is naturally without effect.

An increase of temperature on the other hand accelerates all plant functions, including respiration. In leaves rich in chlorophyll this may not be more than $\frac{1}{20}$ the activity of CO_2 assimilation, but in leaves poor in chlorophyll it represents a large fraction of the CO_2 assimilated. Respiration may be 20 to 40 times as active as 35°C. to 40°C. as at 0°C. , and this would be sufficient to prevent any accelerating action of a rise of temperature upon the photo-synthesis of leaves deficient in chlorophyll being shown.

Willstätter and Stoll's results are, therefore, capable of a simple and natural explanation, and do not support the construction they place upon them.

Theories of Photo-synthesis.

From Jörgensen and Kidd's criticism¹ of the results obtained by Usher and Priestly, by Wager and by myself, it would appear as though the work of these authors was completely antagonistic, and mutually contradictory, and therefore of no value. This is not quite a correct view, as the work of each investigator led on to or directly gave rise to that of the next. Thus Usher and Priestley (l.c.) in supposing that they produced photo-synthesis outside the cell, drew attention to facts which otherwise might have been overlooked, and it was their work which led me to determine that the formaldehyde was a direct product of the photo-oxidation of chlorophyll, and was formed in the absence of carbon dioxide. It was a further investigation of their results which enable me to determine that carbon dioxide decomposes chlorophyll in darkness as well as in light. I was in error in concluding that xanthophyll was one of the products of this decomposition, and this error has been corrected by Willstätter as well as by Jörgensen and Kidd, thus bringing our understanding of the changes possible in photo-synthesis a stage further. The direct action of carbon dioxide is to remove the magnesium from the chlorophyll, forming phaeophytin, and I have shown that if zinc dust is present during this reaction, the zinc steps into the place vacated by the magnesium, forming the stable green zinc chlorophyll.

¹ Proc. Roy. Soc., vol. 89, B., p. 342, 1917.

The removal of the magnesium from chlorophyll by carbon dioxide, and the fact that magnesium will bring about the combination of carbon dioxide and water to formaldehyde suggests at first sight a possible explanation of carbon dioxide assimilation. This action, however, takes place as well in darkness as in light. Further, the magnesium is separated from chlorophyll as the carbonate, and not in the form of the metal, and if any such action was necessary in photo-synthesis, when the latter was active, the bulk of the chlorophyll would exist in the form of phaeophytin, and the leaf should lose its green colour, which is not the case. Carbon dioxide decompose the chlorophyll in heaped grass leaves in darkness, but in living leaves exposed to light in air rich with CO_2 , the chlorophyll remains green. If no carbon dioxide is present, and the illumination is strong, the chlorophyll slowly bleaches, but this is stopped if the supply of carbon dioxide is proportionately increased.

It is more reasonable, therefore, to suppose that the chlorophyll as a whole takes part in photo-synthesis, acting as a light ferment, or lytase enzyme, which, using light energy to draw carbon dioxide and water into its own organisation, breaks down again, liberating them as carbohydrates, and that so long as the supply of CO_2 corresponds to the intensity of the light, and the products are removed, the decomposition and reconstruction of the chlorophyll remain in equilibrium.

There is no difficulty about the decomposition of chlorophyll, but evidence of its regeneration is difficult to obtain.

In a previous paper¹ an account was given of various attempts made to bring about the regeneration of chlorophyll outside the plant. Although indications were obtained suggesting that this might be possible, these were not conclusive. Further attempts in the same direction have so far completely failed. It was frequently observed that in the separation of chlorophyll, alcoholic solutions of xanthophyll, containing the waxy derivatives by precursors of chlorophyll in finely suspended form, when evaporated formed greenish yellow skins, which contained chlorophyll, and left the remaining liquid with little xanthophyll. This happened even when the watery alcoholic liquid showed no trace of chlorophyll under the spectroscope. Further investigations showed, however, that when alcoholic solutions of pure xanthophyll containing a little water are evaporated slowly, the first part of the xanthophyll to

¹ Proc. Roy. Soc. Lond., B., vol. 80, p. 30, 1908.

separate out had a greenish colour owing to its physical condition. When dissolved, it shows no trace of chlorophyll. It was further found that a liquid rich in xanthophyll could contain traces of chlorophyll in finely suspended form without any traces of chlorophyll being visible under the spectroscope. This is partly because owing to the opacity of a liquid containing waxy impurities in finely suspended form, only thin layers can be examined. When such liquids are evaporated, and the green solids which first separate out removed and dissolved, the appearance is given of a production of chlorophyll having taken place. In other observations in which chlorophyll appeared to be regenerated with the agency of zinc dust, the apparent regeneration was due to the formation of the zinc compound of phaeophytin, which closely resembles chlorophyll.

Hitherto the regeneration of chlorophyll from its magnesium containing derivatives, and from xanthophyll or carotin under the action of light, has not been possible outside the living plant. Iwanowski¹ states that solutions of chlorophyll containing carotin or xanthophyll are more stable towards light than chlorophyll alone. This may be partly due to a direct protective action, carotin in particular combining more readily with oxygen than chlorophyll does. According to Iwanowski, however, the protective action is more marked when both carotin and xanthophyll are present. This may possibly be because a certain amount of regeneration of chlorophyll takes place with the aid of light energy from the products of its decomposition and from those of the photo.-oxidation of carotin and xanthophyll.

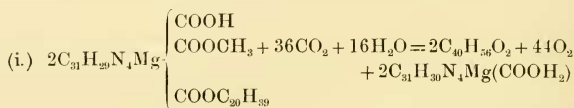
In the case of those plants which can develop chlorophyll in darkness, its building up requires a supply of energy which is either derived from the oxidation of the stored food materials, or which is absorbed directly as heat from outside. It is worthy of note that in all cases the minimum temperature for the formation of chlorophyll is higher than for the formation of carotin or xanthophyll, and plants which can turn green in darkness will do so at a lower temperature in light than they will in darkness.

Until the heats of combustion of chlorophyll, glaucophyllin, phyllophyllin, phytyl, carotin, and xanthophyll are known, it is impossible to discuss the energy changes which may be involved in carbon dioxide assimilation. A large part at least of the energy represented by the carbohydrates produced is undoubtedly light energy, which was absorbed and used in the reconstruction of chloro-

¹ Ber. d. D. Bot. Ges., 1913-14, 31, pp. 600-613.

phyll, and the carbohydrates are probably as much a product of photo-analysis as of photo-synthesis. So long as the supplies of carbon dioxide and light energy are in a certain ratio of equivalency, and the products are continually removed, the chlorophyll is externally stable, though internally labile. In other words, it behaves as an enzyme, and, according to whether we emphasize the source of energy or of material, it might either, to coin a term, be regarded as a lytase enzyme or a carboxidase enzyme.

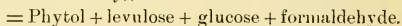
The following equations are put forward not as representing established facts, but as indicating how chlorophyll could act as a photic or lytase enzyme for the conversion of carbon dioxide and water into carbohydrates. The chief difficulty in regard to them is the very large mass reactions which they represent.



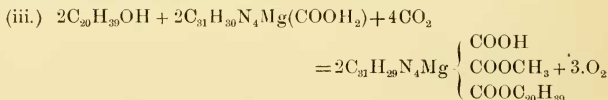
Amorphous chlorophyll + carbon dioxide and water would form xanthophyll (or carotin) oxygen and glaucophyllin.



Carotin (or xanthophyll) + water + oxygen



This reaction would take place in light with the aid of an oxidase enzyme and the excess oxygen from (i.) escapes.



Phytol, glaucophyllin, and carbon dioxide form amorphous chlorophyll and oxygen.

This change would take place in the living plant with the aid of light energy. It makes the total volume of oxygen exhaled equal to the volume of carbon dioxide absorbed.

This suggested cycle would indicate the enzymatic action of chlorophyll in the presence of water, carbon dioxide and light, and might serve as a basis for further investigation. Written in one line the equations would read:—

$40\text{CO}_2 + 40\text{H}_2\text{O} + \text{chlorophyll} + \text{light energy}$



With an excess of carbon dioxide and a deficiency of light, stage 1 would preponderate. With stronger illumination stages 2 and 3 would balance 1. With excessive illumination and a deficiency of carbon dioxide disintegratory photo-oxidation would take place, and the amount of chlorophyll would be reduced.

Summary.

In the assimilation of carbon dioxide chlorophyll acts as a light energizing enzyme, and takes direct part in the cycle of chemical changes, which probably have carotin, xanthophyll, phytyl and glaucophyllins as intermediate products and glucose, levulose, formaldehyde and oxygen as end products. The sugar may be formed directly as well as through the polymerization of formaldehyde.

A large part of the energy represented by the carbohydrate products is absorbed during the reconstruction of the chlorophyll molecule.

Carbon dioxide decomposes extracted chlorophyll both in light and in darkness. The earlier supposition that xanthophyll was one of the products has not been sustained. In the presence of zinc dust, the zinc takes the place of the magnesium, and the chlorophyll remains green as a stable zinc chlorophyll.

Apart from its protective function, carotin seems to be especially important as providing during its photo-oxidation or partial disintegration, the massive hydrocarbon combination in the phytyl radicle of chlorophyll whose addition is necessary to convert the dicarboxylic glaucophyllin into the tricarboxylic chlorophyll. Xanthophyll can be reduced to carotin by the aid of metallic reductases, but no oxidases have been found capable of converting carotin into xanthophyll. The oxidation of these substances in darkness or in feeble light differs in certain respects from that taking place in intense light.

The oxidation of rhodophyllin, chlorophyll, xanthophyll, and carotin is more rapid at high temperatures than at low ones, and the rates of oxidation are in the order given, carotin being most readily oxidized.

A rapid method is described of polymerizing formaldehyde to sugar, which has a definite end reaction, and yields calcium and sodium tartrates as by-products.